Product Information:

Fast Lysis-PCR Genotyping Kit

Kit contains: Reagent-A (10ml); Reagent-B (500ul); Reagent-C (10ml);
2x PCR Mastermix (1ml); DNA precipitation solution (10ml);;
Catalog: GT-001PD
Sizes: 100 extractions
Storage : -20°C

Description:
Fast lysis-PCR genotyping reagents contain a combination of enzyme(s), detergents, and PCR required reagents that lysate the mouse/rat tissues (tails, ears, yolk sacs) within 30 minutes and directly used to run PCR reactions without any further DNA purification.

Procedure:

1. Prepare 0.2-0.3cm mouse tail biopsy sample in a 0.5ml or 1.5ml microcentrifuge tube.
2. Add 100ul reagent-A and 5ul reagent-B into the sample tube. Incubate at 60ºC for 10 minutes .
3. Place the tube in a PCR machine (or water bath or block) and incubate at 95ºC for 20 minutes.
4. Centrifuge at 12,000 xg for 2 minutes at 4ºC and transfer the lysate supernatant into a clean tube.
5. Add 100ul reagent-C into the lysate supernatant and mix by vortexing.
6. Pipette 2-10ul lysates into a 20ul PCR mastermix and run PCR/Real-Time PCR at thermal cyclers.

Suggested PCR Protocol:

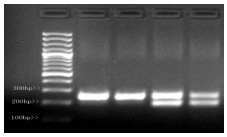
I. Preparation of PCR Master Mix
for a single reaction (total volume: 20uL) in a 0.2 or 0.5mL microtube.

Component	Volume (µL)	Final Concentration
Lysate supernatant	2-10	determined by user
2x PCR mastermix	10	1x
Forward primer (5µM)	1	200nM
Reverse primer (5µM)	1	200nM
PCR grade water	up to 20 µL	

II. Setup typical thermal cycling parameters

Enzyme activation step:	95°C	5 minutes
25-40 cycles:		
Denaturation	95°C	30 seconds
Annealing	X°C	30 seconds dependent on Tm of primers
Extension	72°C	30 seconds (1min per kb amplicon)
Hold at 4-8°C		

III. Figure:



PCR analysis of genomic DNA extracts from mouse tail biopsy at 0.2-0.3 cm, using Fast Lysis-PCR genotyping kit (GT-001). Genomic DNA in 2ul lysate supernatant was directly amplified in a 20ul PCR reaction for 35 cycles.

Technical Tips:

1. Ensure the tissue samples (tail, ear or yolk sac) are completely submerged in the reagents. (Use roughly proportional volume of reagents for different sized samples)

	mouse tail (0.2-0.3cm)	ear(0.2cm)	yolk sac (E8.5)
Reagent-A (ul):	100	80	50
Reagent-B (ul):	5	4	2.5
Reagent-C (ul):	100	80	50

2. Tissues will not completely digested at the end of the incubation. This is normal and not influence PCR performance. Undigested tissues were stored at -20°C, used for other applications or further genotypings. If necessary, longer incubation or overnight at step 2. can completely digest the tissue sample and no loss of efficacy.
3. Lysates are stable at 4°C for at least 6 months and at room temperature for 1-2 weeks. For longer-term storage at room temperature, it is necessary to further purify the crude lysates, using the following procedure: add NaCl to a final concentration of 250mM, and then add 0.7 volume of pure ethanol to precipitate DNA, spin down DNA at 4°C for 3 minutes, discard supernatant and dissolve DNA in 50ul TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA).
4. The kit may be used for other genomic DNA extractions from various animal tissues, hair shafts, toes or saliva.
5. Additional information: (An new technical procedure for DNA precipitation)

Procedure:

1. Prepare 0.2-0.3cm mouse tail biopsy sample in a 0.5ml or 1.5ml microcentrifuge tube.
2. Add 100ul reagent-A and 5ul reagent-B into the sample tube. Incubate at 60ºC for 10 minutes .
   If necessary, stroke the tissues with pestle can completely digest the tissue sample to obtain higher DNA yields.
3. Place the tube in a PCR machine (or water bath or block) and incubate at 95ºC for 20 minutes.
4. Centrifuge at 12,000 xg for 2 minutes at 4ºC and transfer the lysate supernatant into a clean tube.
5. Add 100ul reagent-C into the lysate supernatant and mix by vortexing.
6. Centrifuge at 3,000 xg for 5 minutes at 4ºC and transfer the lysate supernatant into a clean tube.
7. Add 100ul DNA precipitation solution (Cat.#: PS-01D) into the lysate supernatant. Mix well and centrifuge at 12,000 xg for 10 minutes at 4ºC. Aspirate liquid and simply wash pellet with 80% ethanol for 2 times (don't resuspend the DNA pellets). Air-dry pellet for 5-10 minutes and dissolve DNA in 50ul TE buffer or distilled water. Measure the DNA Concentration with 260/280nm and store DNA at room temperature or at 4ºC.
8. Pipette 1-2ul lysates into a 20ul PCR Master Mixture and run PCR/Real-Time PCR at thermal cyclers.

Precautions and Disclaimer:

This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.